

[0038]

As clearly shown in Table 1, each type of recombinant cell achieved relatively efficient accumulation of 5 to 50 wt % polyester using 2 % glucose as a carbon source. *Pseudomonas* sp. strain 61-3 (*phbC::tet*) produced copolymer polyester P(3HB-co-3HA) having a 3HB molar composition of 36 mol%, while the strain ASc22 with the introduced *phaC1* gene produced polyester having a 3HB molar composition of approximately 64 mol %, slightly higher than that of the former polyester. On the other hand, the strains BB49, KSc46, and KSc54, into which *phbA* and *phbB* genes derived from *Ralstonia eutropha* had been introduced, produced polyester having as high as 81 to 92 mol % of a 3HB molar composition. Moreover, these strains accumulated polyester in the cells at a high intracellular accumulation ratio of 37 to 45 wt %. Unlike P(3HB), copolymer polyester P(3HB-co-3HA) with a high 3HB molar composition has better flexibility and good impact resistance. Therefore, it is concluded that more practical biodegradable plastic can be produced at higher efficiency by using the above described strains.

[0039]

[Effects]

A method of this invention is to synthesize copolymer polyester P(3HB-co-3HA) comprising 3-hydroxyalkanoic acid units with a carbon number of 4 to 12 and having a high 3HB molar composition. Such a polyester is useful because it has good thermal stability and mold ability, and can be made into biodegradable plastic with impact resistance better than that of P(3HB).

[0040]

[Sequence Listing]

Sequence Listing

<110> The Institute of Physical and Chemical Research ; Japan Science and Technology

<120> The method of production of copolymerized polyester

<130> RJH11-020

<160> 11

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<222> (1).. (1677)

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Phe Gly Lys Ser Lys Leu Gln Pro Glu Ser Asp Asp Arg Arg Phe Asn

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Leu Ser Glu Gln Asp Ile Asn Arg Ala His Phe Val Ile Thr Leu Met

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Thr	Asn Asp His Ile Thr Pro Trp Lys Ser Cys Tyr Lys Ser Ala Gln		
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Leu	Phe Gly Gly Lys Val Glu Phe Val Leu Ser Ser Ser Gly His Ile		
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Gln	Ser Ile Leu Asn Pro Pro Gly Asn Pro Lys Ser Arg Tyr Met Thr		
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Ser Val Val Phe Arg Asn Glu Leu Leu Glu Leu Met Gln Tyr Lys Pro				
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			Pro		
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					255

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Val Ile Lys Ala Ala Leu Glu Arg Ala Gly Val Lys Pro Glu Gln Val	
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agc gaa gtc atc atg ggc cag gtg ctg acc gcc ggt tgc ggc cag aac	192
Ser Glu Val Ile Met Gly Gln Val Leu Thr Ala Gly Ser Gly Gln Asn	
50 55 60	
ccc gca cgc cag gcc gcg atc aag gcc ggc ctg ccg gcg atg gtg ccg	240
Pro Ala Arg Gln Ala Ala Ile Lys Ala Gly Leu Pro Ala Met Val Pro	
65 70 75 80	
gcc atg acc atc aac aag gtg tgc ggc tgc ggc ctg aag gcc gtg atg	288
Ala Met Thr Ile Asn Lys Val Cys Gly Ser Gly Leu Lys Ala Val Met	
85 90 95	
ctg gcc gcc aac gcg atc atg gcg ggc gac gcc gag atc gtg gtg gcc	336
Leu Ala Ala Asn Ala Ile Met Ala Gly Asp Ala Glu Ile Val Val Ala	
100 105 110	
ggc ggc cag gaa aac atg agc gcc gcc ccg cac gtg ctg ccg ggc tgc	384
Gly Gly Gln Glu Asn Met Ser Ala Ala Pro His Val Leu Pro Gly Ser	
115 120 125	
cgc gat ggt ttc cgc atg ggc gat gcc aag ctg gtc gac acc atg atc	432
Arg Asp Gly Phe Arg Met Gly Asp Ala Lys Leu Val Asp Thr Met Ile	
130 135 140	
gtc gac ggc ctg tgg gac gtg tac aac cag tac cac atg ggc atc acc	480
Val Asp Gly Leu Trp Asp Val Tyr Asn Gln Tyr His Met Gly Ile Thr	
145 150 155 160	
gcc gag aac gtg gcc aag gaa tac ggc atc aca cgc gag gcg cag gat	528
Ala Glu Asn Val Ala Lys Glu Tyr Gly Ile Thr Arg Glu Ala Gln Asp	
165 170 175	
gag ttc gcc gtc ggc tgc cag aac aag gcc gaa gcc gcg cag aag gcc	576
Glu Phe Ala Val Gly Ser Gln Asn Lys Ala Glu Ala Ala Gln Lys Ala	
180 185 190	
ggc aag ttt gac gaa gag atc gtc ccg gtg ctg atc ccg cag cgc aag	624
Gly Lys Phe Asp Glu Glu Ile Val Pro Val Leu Ile Pro Gln Arg Lys	

195	200	205	
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Gly Asp Pro Val Ala Phe Lys Thr Asp Glu Phe Val Arg Gln Gly Ala			
210	215	220	
acg ctg gac agc atg tcc ggc ctc aag ccc gcc ttc gac aag gcc ggc			720
Thr Leu Asp Ser Met Ser Gly Leu Lys Pro Ala Phe Asp Lys Ala Gly			
225	230	235	240
acg gtg acc gcg gcc aac gcc tcg ggc ctg aac gac ggc gcc gcc gcg			768
Thr Val Thr Ala Ala Asn Ala Ser Gly Leu Asn Asp Gly Ala Ala Ala			
245	250	255	
gtg gtg gtg atg tcg gcg gcc aag gcc aag gaa ctg ggc ctg acc ccg			816
Val Val Val Met Ser Ala Ala Lys Ala Lys Glu Leu Gly Leu Thr Pro			
260	265	270	
ctg gcc acg atc aag agc tat gcc aac gcc ggt gtc gat ccc aag gtg			864
Leu Ala Thr Ile Lys Ser Tyr Ala Asn Ala Gly Val Asp Pro Lys Val			
275	280	285	
atg ggc atg ggc ccg gtg ccg gcc tcc aag cgc gcc ctg tcg cgc gcc			912
Met Gly Met Gly Pro Val Pro Ala Ser Lys Arg Ala Leu Ser Arg Ala			
290	295	300	
gag tgg acc ccg caa gac ctg gac ctg atg gag atc aac gag gcc ttt			960
Glu Trp Thr Pro Gln Asp Leu Asp Leu Met Glu Ile Asn Glu Ala Phe			
305	310	315	320
gcc gcg cag gcg ctg gcg gtg cac cag cag atg ggc tgg gac acc tcc			1008
Ala Ala Gln Ala Leu Ala Val His Gln Gln Met Gly Trp Asp Thr Ser			
325	330	335	
aag gtc aat gtg aac ggc ggc gcc atc gcc atc ggc cac ccg atc ggc			1056
Lys Val Asn Val Asn Gly Gly Ala Ile Ala Ile Gly His Pro Ile Gly			
340	345	350	
gcg tcg ggc tgc cgt atc ctg gtg acg ctg ctg cac gag atg aag cgc			1104

Ala Ser Gly Cys Arg Ile Leu Val Thr Leu Leu His Glu Met Lys Arg
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cgt gac gcg aag aag ggc ctg gcc tcg ctg tgc atc ggc ggc ggc atg 1152
Arg Asp Ala Lys Lys Gly Leu Ala Ser Leu Cys Ile Gly Gly Gly Met
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<211> 393

<212> PRT

<213> *Ralstonia eutropha*

<400> 6

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20 25 30
Val Ile Lys Ala Ala Leu Glu Arg Ala Gly Val Lys Pro Glu Gln Val
35 40 45
Ser Glu Val Ile Met Gly Gln Val Leu Thr Ala Gly Ser Gly Gln Asn
50 55 60
Pro Ala Arg Gln Ala Ala Ile Lys Ala Gly Leu Pro Ala Met Val Pro
65 70 75 80
Ala Met Thr Ile Asn Lys Val Cys Gly Ser Gly Leu Lys Ala Val Met
85 90 95
Leu Ala Ala Asn Ala Ile Met Ala Gly Asp Ala Glu Ile Val Val Ala
100 105 110

Gly	Gly	Gln	Glu	Asn	Met	Ser	Ala	Ala	Pro	His	Val	Leu	Pro	Gly	Ser
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Arg	Asp	Gly	Phe	Arg	Met	Gly	Asp	Ala	Lys	Leu	Val	Asp	Thr	Met	Ile
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Val	Asp	Gly	Leu	Trp	Asp	Val	Tyr	Asn	Gln	Tyr	His	Met	Gly	Ile	Thr
145				150				155				160			
Ala	Glu	Asn	Val	Ala	Lys	Glu	Tyr	Gly	Ile	Thr	Arg	Glu	Ala	Gln	Asp
165				170				175							
Glu	Phe	Ala	Val	Gly	Ser	Gln	Asn	Lys	Ala	Glu	Ala	Ala	Gln	Lys	Ala
180				185				190							
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210				215				220							
Thr	Leu	Asp	Ser	Met	Ser	Gly	Leu	Lys	Pro	Ala	Phe	Asp	Lys	Ala	Gly
225				230				235				240			
Thr	Val	Thr	Ala	Ala	Asn	Ala	Ser	Gly	Leu	Asn	Asp	Gly	Ala	Ala	Ala
245				250				255							
Val	Val	Val	Met	Ser	Ala	Ala	Lys	Ala	Lys	Glu	Leu	Gly	Leu	Thr	Pro
260				265				270							
Leu	Ala	Thr	Ile	Lys	Ser	Tyr	Ala	Asn	Ala	Gly	Val	Asp	Pro	Lys	Val
275				280				285							
Met	Gly	Met	Gly	Pro	Val	Pro	Ala	Ser	Lys	Arg	Ala	Leu	Ser	Arg	Ala
290				295				300							
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Ala	Ala	Gln	Ala	Leu	Ala	Val	His	Gln	Gln	Met	Gly	Trp	Asp	Thr	Ser
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<211> 738

<212> DNA

<213> *Ralstonia eutropha*

<220>

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Thr Ala Ile Cys Gln Arg Leu Ala Lys Asp Gly Phe Arg Val Val Ala	
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ggt tgc ggc ccc aac tcg ccg cgc cgc gaa aag tgg ctg gag cag cag	144
Gly Cys Gly Pro Asn Ser Pro Arg Arg Glu Lys Trp Leu Glu Gln Gln	
35 40 45	
aag gcc ctg ggc ttc gat ttc att gcc tcg gaa ggc aat gtg gct gac	192
Lys Ala Leu Gly Phe Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp	

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Trp Asp Ser Thr Lys Thr Ala Phe Asp Lys Val Lys Ser Glu Val Gly			
65	70	75	80
gag gtt gat gtg ctg atc aac aac gcc ggt atc acc cgc gac gtg gtg	288		
Glu Val Asp Val Leu Ile Asn Asn Ala Gly Ile Thr Arg Asp Val Val			
85	90	95	
ttc cgc aag atg acc cgc gcc gac tgg gat gcg gtg atc gac acc aac	336		
Phe Arg Lys Met Thr Arg Ala Asp Trp Asp Ala Val Ile Asp Thr Asn			
100	105	110	
ctg acc tcg ctg ttc aac gtc acc aag cag gtg atc gac ggc atg gcc	384		
Leu Thr Ser Leu Phe Asn Val Thr Lys Gln Val Ile Asp Gly Met Ala			
115	120	125	
gac cgt ggc tgg ggc cgc atc gtc aac atc tcg tcg gtg aac ggg cag	432		
Asp Arg Gly Trp Gly Arg Ile Val Asn Ile Ser Ser Val Asn Gly Gln			
130	135	140	
aag ggc cag ttc ggc cag acc aac tac tcc acc gcc aag gcc ggc ctg	480		
Lys Gly Gln Phe Gly Gln Thr Asn Tyr Ser Thr Ala Lys Ala Gly Leu			
145	150	155	160
cat ggc ttc acc atg gca ctg gcg cag gaa gtg gcg acc aag ggc gtg	528		
His Gly Phe Thr Met Ala Leu Ala Gln Glu Val Ala Thr Lys Gly Val			
165	170	175	
acc gtc aac acg gtc tct ccg ggc tat atc gcc acc gac atg gtc aag	576		
Thr Val Asn Thr Val Ser Pro Gly Tyr Ile Ala Thr Asp Met Val Lys			
180	185	190	
gcg atc cgc cag gac gtg ctc gac aag atc gtc gcg acg atc ccg gtc	624		
Ala Ile Arg Gln Asp Val Leu Asp Lys Ile Val Ala Thr Ile Pro Val			
195	200	205	
aag cgc ctg ggc ctg ccg gaa gag atc gcc tcg atc tgc gcc tgg ttg	672		

Lys Arg Leu Gly Leu Pro Glu Glu Ile Ala Ser Ile Cys Ala Trp Leu
 210 215 220
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<210> 8

<211> 246

<212> PRT

<213> *Ralstonia eutropha*

<400> 8

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 35 40 45
 Lys Ala Leu Gly Phe Asp Phe Ile Ala Ser Glu Gly Asn Val Ala Asp
 50 55 60
 Trp Asp Ser Thr Lys Thr Ala Phe Asp Lys Val Lys Ser Glu Val Gly
 65 70 75 80
 Glu Val Asp Val Leu Ile Asn Asn Ala Gly Ile Thr Arg Asp Val Val
 85 90 95
 Phe Arg Lys Met Thr Arg Ala Asp Trp Asp Ala Val Ile Asp Thr Asn
 100 105 110

Leu Thr Ser Leu Phe Asn Val Thr Lys Gln Val Ile Asp Gly Met Ala
 115 120 125
 Asp Arg Gly Trp Gly Arg Ile Val Asn Ile Ser Ser Val Asn Gly Gln
 130 135 140
 Lys Gly Gln Phe Gly Gln Thr Asn Tyr Ser Thr Ala Lys Ala Gly Leu
 145 150 155 160
 His Gly Phe Thr Met Ala Leu Ala Gln Glu Val Ala Thr Lys Gly Val
 165 170 175
 Thr Val Asn Thr Val Ser Pro Gly Tyr Ile Ala Thr Asp Met Val Lys
 180 185 190
 Ala Ile Arg Gln Asp Val Leu Asp Lys Ile Val Ala Thr Ile Pro Val
 195 200 205
 Lys Arg Leu Gly Leu Pro Glu Glu Ile Ala Ser Ile Cys Ala Trp Leu
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 Gly Gly Leu His Met Gly
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<210> 9

<211> 542

<212> DNA

<213> Pseudomonas sp. strain 61-3

<400> 9

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 aaattgacct tcgacgacgg ccatgacagc ggctgttca cctgggatta tctgtaccaa 180
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gcctgcttgc gaaaaaaatt aaactcgggt aaccaatgga gctggcaagt tccctgcagt 420
gctctctgaa ctagaaagca acgttctgca attaacggtc acccgagcag tagtacctgg 480
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<210> 10

<211> 841

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<213> *Ralstonia eutropha*

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ccacgtagag ccagccaatg gccacgaigt acatcaaaaa ttcatccttc tcgcctatgc 420
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gccgagcgcg attcccccat tgacagcgcg tgcgttgcaa ggcaacaatg gactcaaatg 540
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cctccccgtt tgggggaggg gcaagccggg tccattcgga tagcatctcc ccatgcaaag 780
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<210> 11

<211> 292

<212> DNA

<213> *Ralstonia eutropha*

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gcagccagca ccatgttcgt gcagcgcggc cctcgcgggg gcgaggctgc ag          292
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[Brief Description of the drawings]

[Fig.1] Fig. 1 shows a synthetic pathway of polyester.

[Fig.2] Fig. 2 shows steps to construct a recombinant vector.

[Fig.3] Fig. 3 shows a structure of the recombinant vector used for transformation of *Pseudomonas* sp. strain 61-3 (*phbC::tet*).